Urea-PAGE patterns of PDO Évora cheese made with *Cynara cardunculus* L. ecotypes during ripening

Pinheiro, Cristina.*, Lamy, E., Rodrigues, L., Garrido, A. L., Freitas, S., Alvarenga, N. B., Dias, J., Martins, A. P. L. and Duarte, F.

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Dubrovnik, Croatia, 27th to 31st August 2018

- Urea-PAGE patterns of PDO Évora cheese
- Effect of *Cynara cardunculus* L ecotypes on Évora cheese proteolysis

What?

… Portuguese and Spanish ewe's cheese made with the aqueous extract of *Cynara cardunculus* L. dried flowers …

Cheese characteristics

Why?

Évora PDO cheese made with 3 ecotypes, analysed during ripening

How?

Évora local cheesemaker | ICAAM Lab.

Where?

ValBiotecCynara team (ALT20-03-0145-FEDER-000038)

Who?

Background

Cynara cardunculus L.

- The most widespread species of *Cynara* genus, also know as cardoon;
- Native to the Mediterranean region;
- Grows naturally in harsh habitat conditions;
- Acid or aspartic proteinases.

Dried flowers have been employed successfully in the manufacture of Spanish and Portuguese cheeses

Background – Research Papers

***Cynara cardunculus:* Use in Cheesemaking and Pharmaceutical Applications**

Cristina Conceição, Pedro Martins, Nuno Alvarenga, João Dias, Elsa Lamy, Lúcia Garrido, Sandra Gomes, Sofia Freitas, Ana Belo, Teresa Brás, Ana Paulino and Maria F. Duarte

https://cdn.intechopen.com/pdfs/61288.pdf

Selected Cardoon (*Cynara cardunculus* L.) Genotypes Suitable for PDO Cheeses in Mediterranean Regions

Paulo Barracosa, Nuno Rosa, Marlene Barros, Euclides Pires

https://onlinelibrary.wiley.com/doi/abs/10.1002/cbdv.201800110

Serra da Estrela Cheese: evaluation of the thistle ecotype on the physical, chemical and sensorial properties

Marlene I. C. Tenreiro[1], Raquel P. F. Guiné[2*], Paulo Barracosa[3], Ana Cristina Correia[1], Paula M. R. Correia[2]

https://bibliotecadigital.ipb.pt/bitstream/10198/12390/3/Poster%20Int.%20136.pdf

Proteolytic effect of *Cynara cardunculus* rennet for use in the elaboration of 'Torta del Casar' cheese

Elena Ordiales[1], Maria José Benito[2*], Alberto Martin[2], Margarita Fernández[2], Alejandro Hernández[2] and Maria de Guia Córdoba[2]

https://www.ncbi.nlm.nih.gov/pubmed/24063288

Nutrition & Food Science

Effect of different thistle flower ecotypes as milk-clotting in Serra da Estrela cheese

Paula Correia, André Vítor, Marlene Tenreiro, Ana Cristina Correia, João Madanelo, Raquel Guiné,

https://www.emeraldinsight.com/doi/abs/10.1108/NFS-12-2015-0157?journalCode=nfs

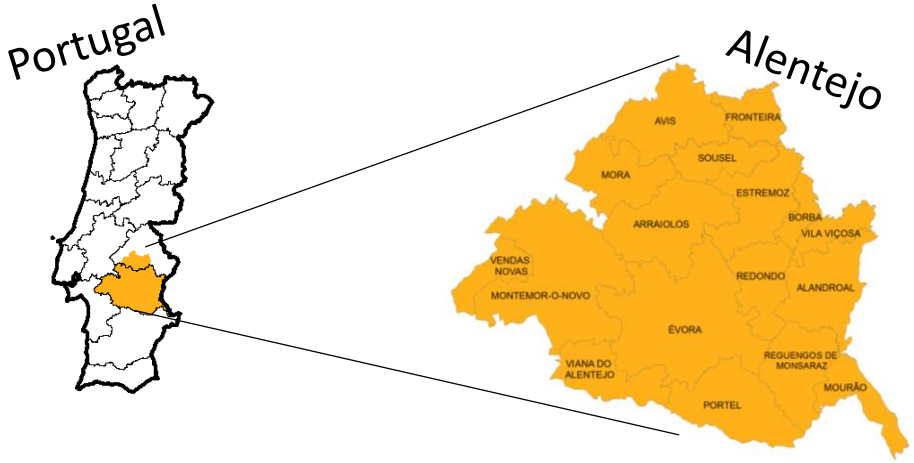
Cardoon-based rennets for cheese production.

Almeida CM[1], Simões I[2,3,4].

https://link.springer.com/article/10.1007/s00253-018-9032-3

Background – Évora Cheese

Évora cheese



- PDO cheese since 1994;
- Manufactured from raw ewe's milk in the Alentejo region;
- At least 30 days of ripening;
- Flavour is characterized with:
  - High intensity of piquant;
  - Salty;
  - Slightly acidic.

REVIEW ARTICLE

Cheese: Food Perception and Food Choice

Lénia Rodrigues[1], Maria Machado[1,2] and Cristina Pinheiro[1,2,*]

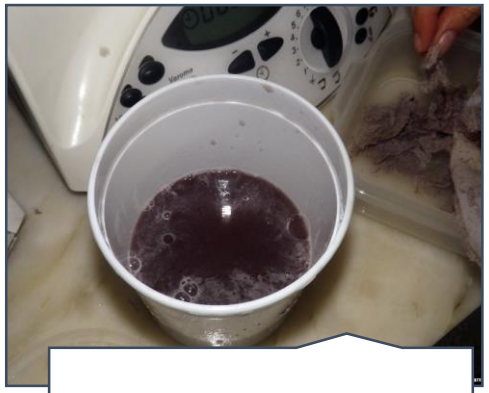
https://www.ncbi.nlm.nih.gov/pubmed/29984675

Recent Pat Food Nutr Agric. 2018 Jul 4. doi: 10.2174/2212798410666180705092257.

Cheesemaking process

Collecting the flowers ecotypes

Dried flowers



Aqueous extract

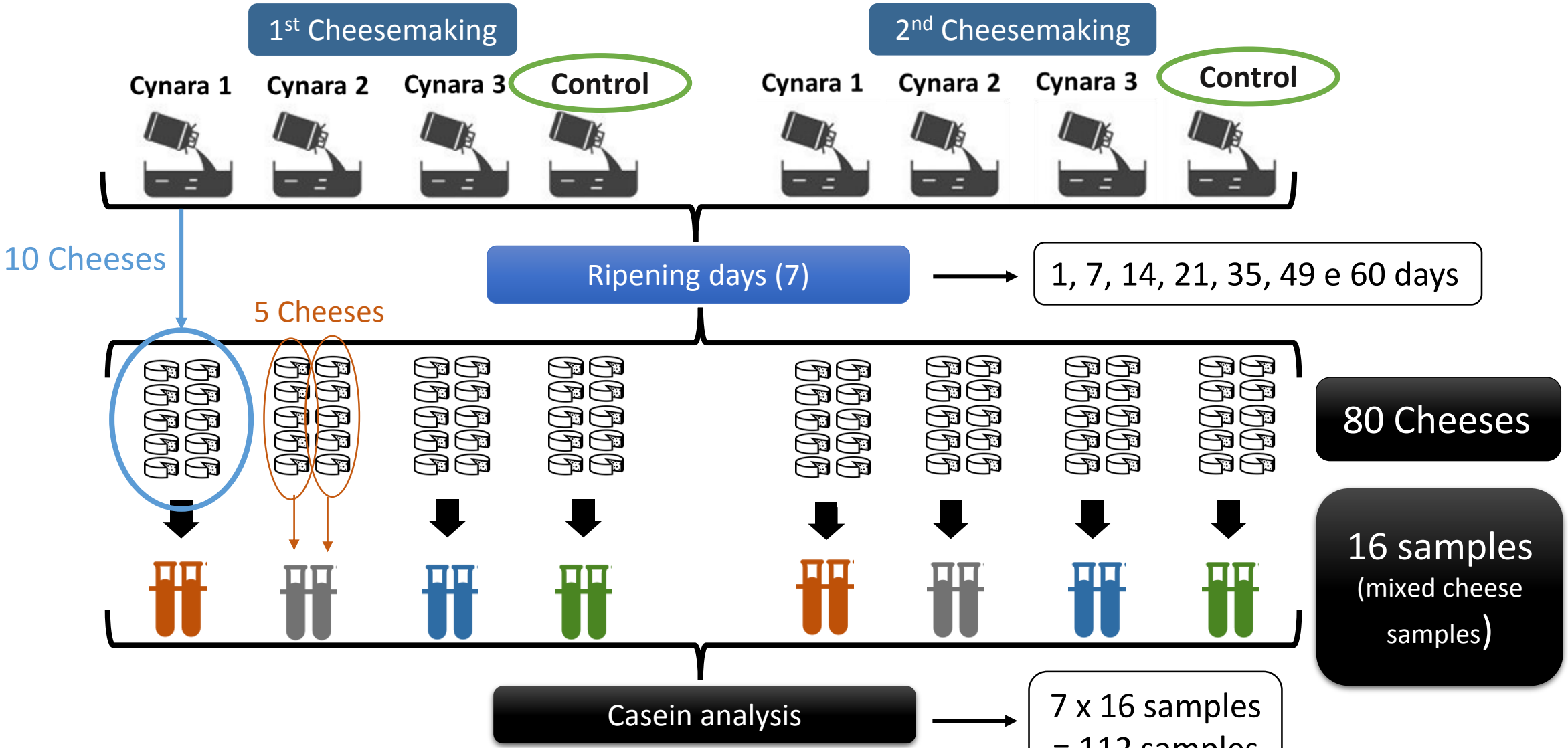
Coagulation

Cheesemaking

Curd poured into cylindrical multi-moulds

Ripening

Material & Methods



Material & Methods - Lab Protocols

Protocol of **casein extraction** was according to Ordiales *et al*., 2013 with some modifications applied

1g of samples + 10 ml of 1M ammonia-acetate buffer

↓

Centrifugation at **6000*g***, 2°C

↓

Adding 10 ml of 1mM ammonia-acetate buffer

↓

Centrifugation **at 6000*g***, 2°C

} 2X

↓

Samples are washed with 5ml acetone (90%) — 2X

↓

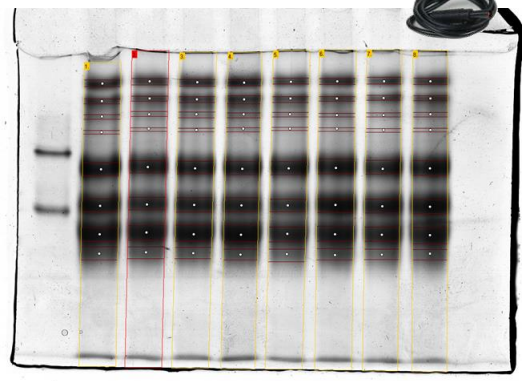
Precipitate obtained = **casein fraction**

Protocol of **urea-PAGE** as described by Andrews, 1993 with the modifications described by Veloso *et al*., 2002.

4% acrylamide in stacking gels

+

10% acrylamide in resolving gels



Gels were scanned and analysed

↓

Each band was quantified as percentage of the total volume

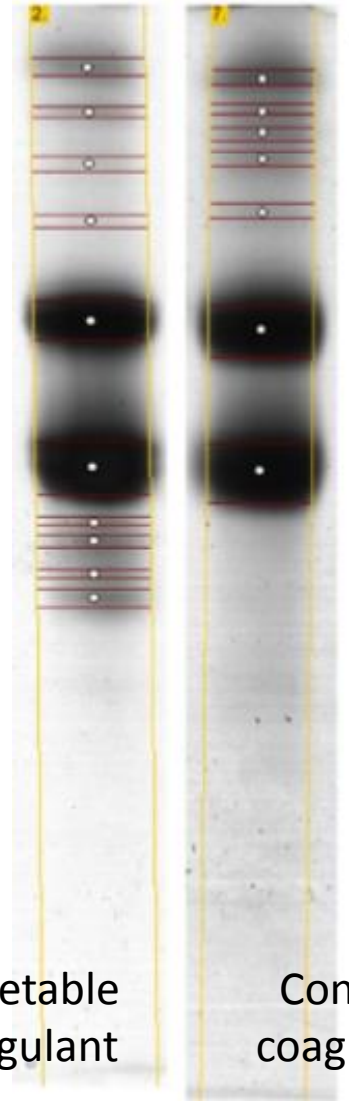
Results and discussion

1.Casein electrophoretic fractions of Évora cheese

1.urea-PAGE patterns profile between the coagulation agents during ripening

Casein electrophoretic fractions of Évora cheese

Identified bands:
From A to L



γ-caseínas

β-caseínas

α_S-caseínas

pré-α_S-caseínas

Acording to…

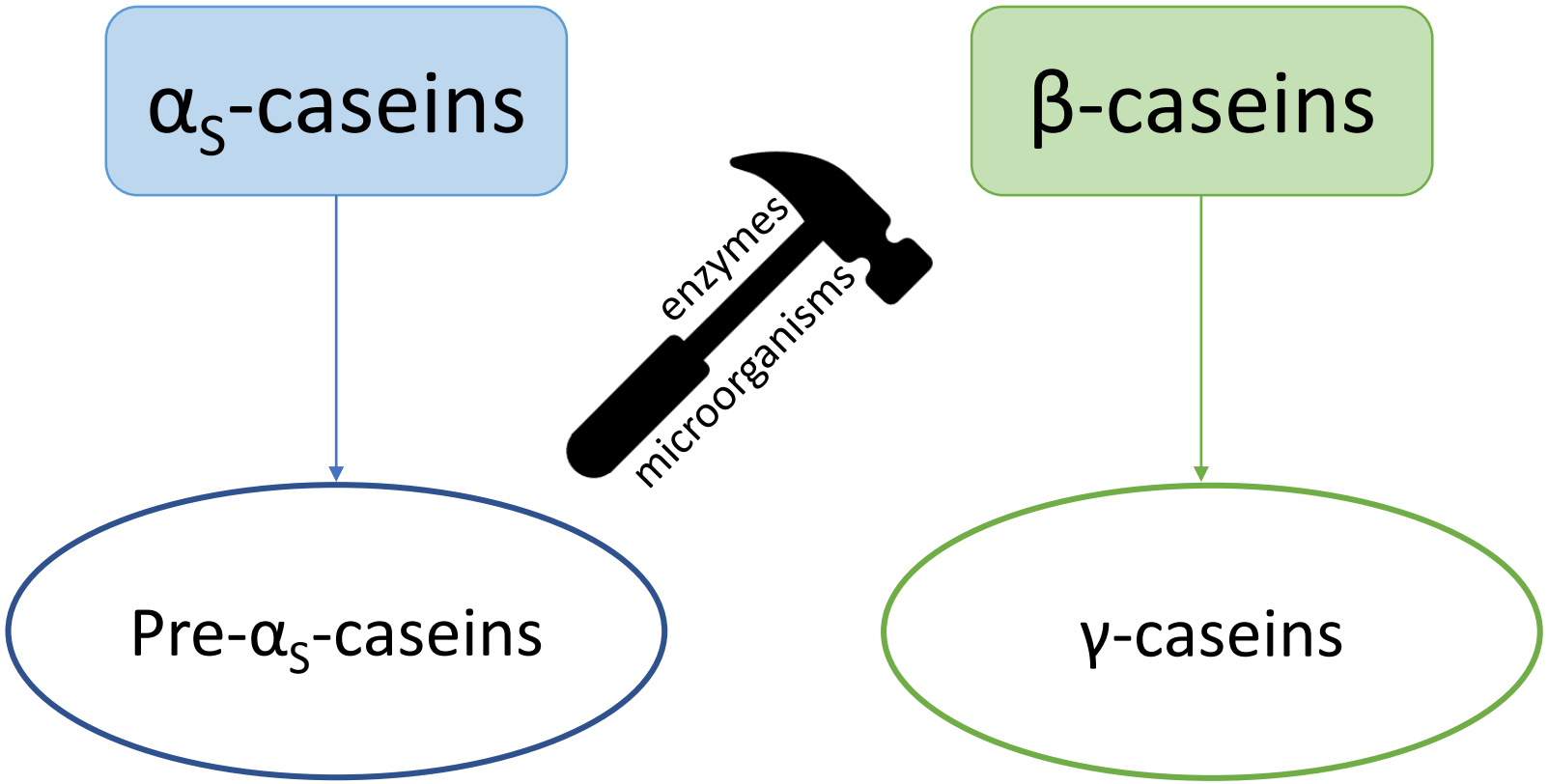
Marcos et al. (1979)

Vegetable coagulant

Control coagulant

Casein degradation through ripening

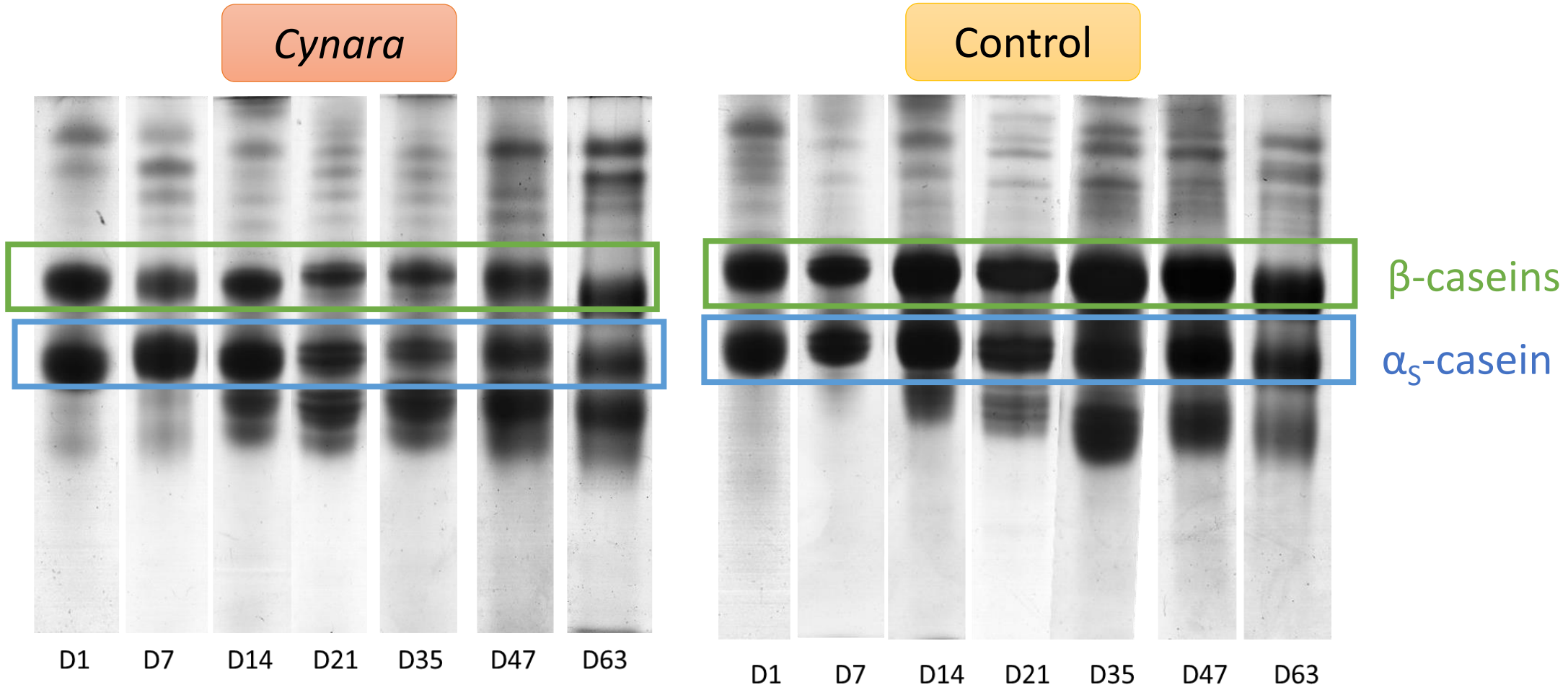
Marked as the main caseins of cheese



Proteolysis

- The degradation of α_S-caseins and β-caseins was observed and the percentage of the total amount of the respective casein initially present in the curd was calculated

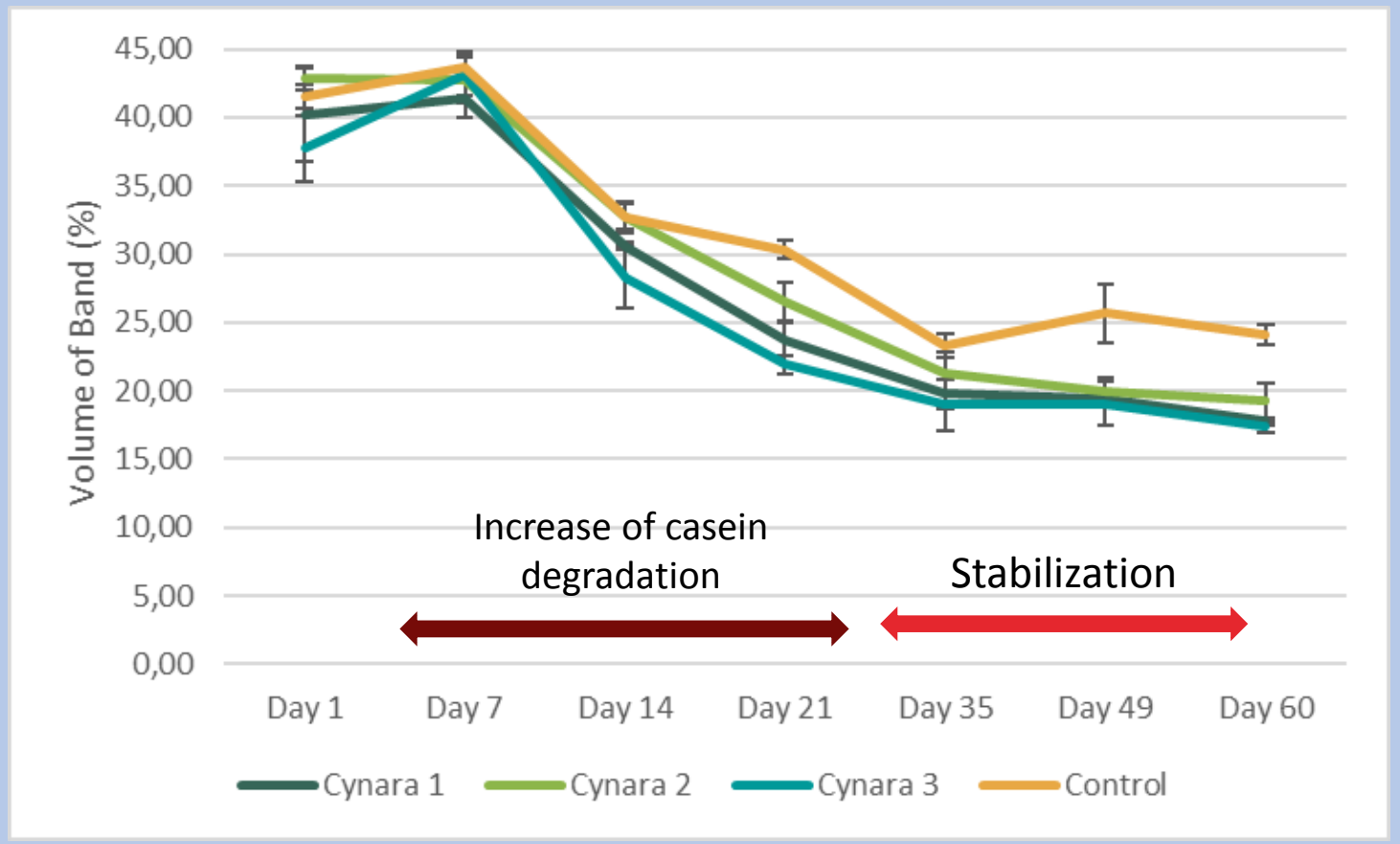
Urea-PAGE patterns profile during ripening



Urea-polyacrylamide gel electrophoretograms of the main caseins of Évora PDO cheese made using *Cynara cardunculus* L. coagulant (Cynara) and animal rennet(Control) at 1, 7, 14, 21, 35, 47 and63 days of ripening.

Urea-PAGE patterns profile during ripening and degradation ratio

α_S-casein



Volume of band (%) ↓

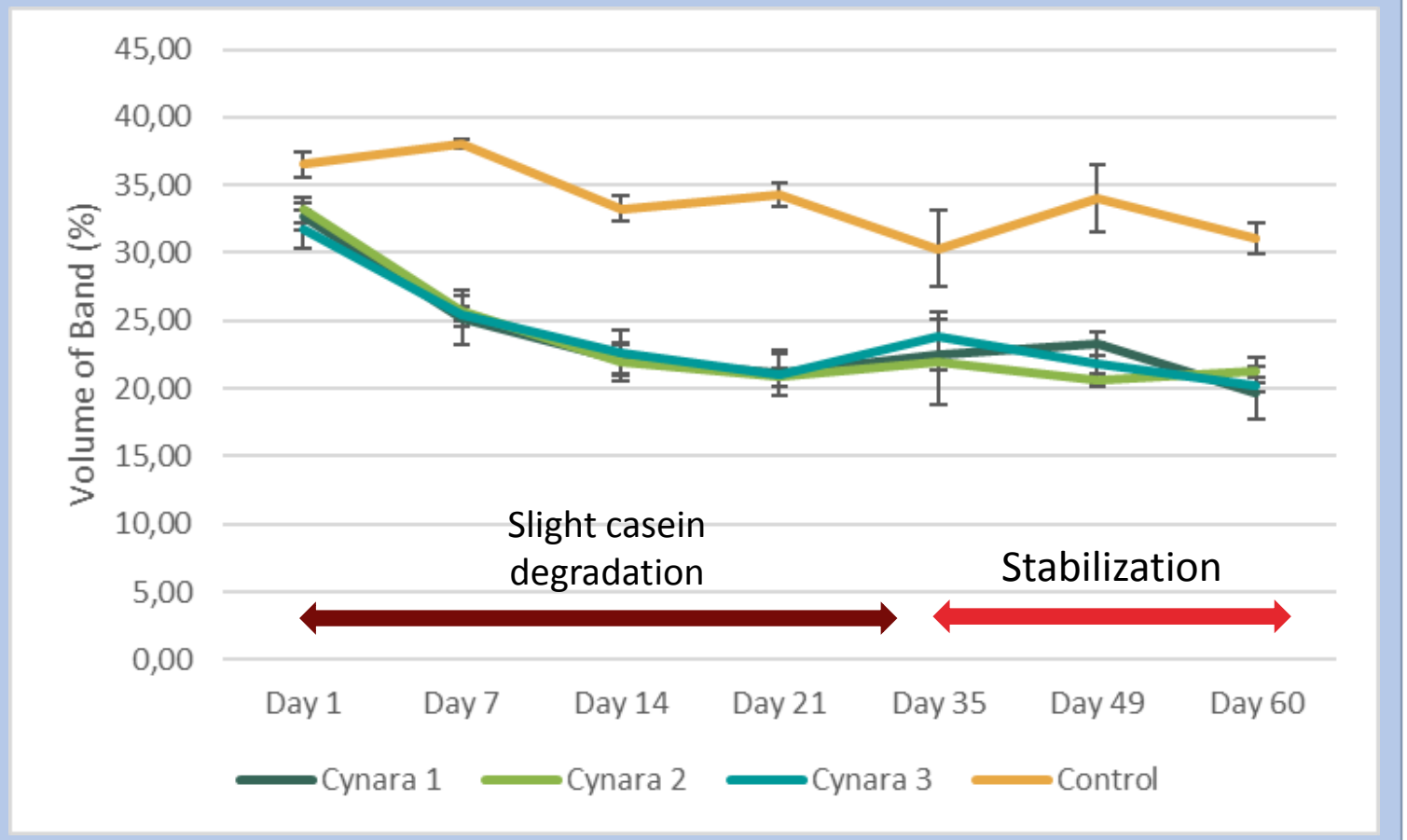
=

Degradation (%) ↑

Coagulant agente	Degradation ratio - 1 to 60 days (%)
Cynara 1	55, 76 [a]
Cynara 2	54, 99 [a]
Cynara 3	53, 95 [a]
Control	41, 86 [b]

Urea-PAGE patterns profile during ripening and degradation ratio

β-casein



Volume of band (%) ↓

=

Degradation (%) ↑

Coagulant agente	Degradation ratio - 1 to 60 days (%)
Cynara 1	39, 81[a]
Cynara 2	35,72[a]
Cynara 3	36,27[a]
Control	14,95[b]

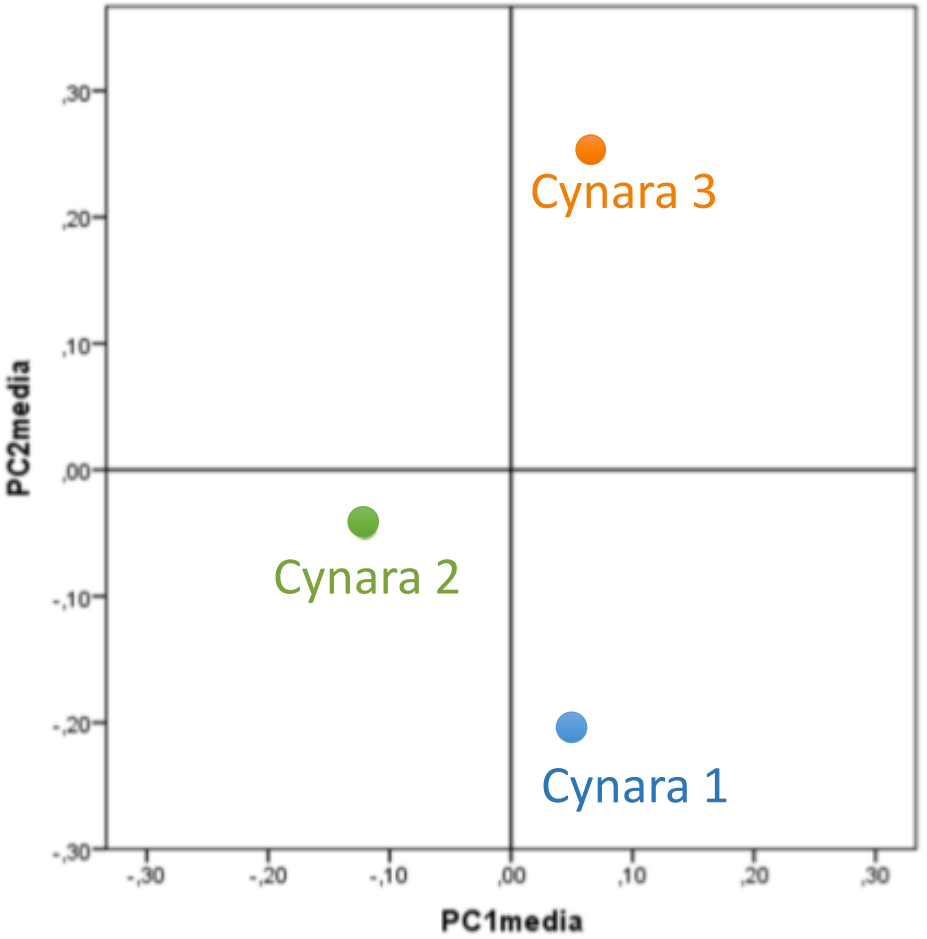
Principal component analysis

- **PC1**

Cynara 2 is separated by the other two because of the **degradation of α_s-caseína (↓ lower)**;

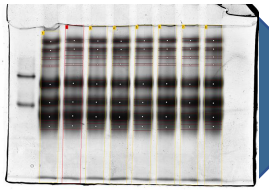
- **PC2**

Cynara 3 is separated by the other two by the **products of degradation of β-caseín (↑ higher)**

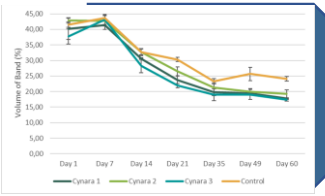


Conclusions

- 11 bands were identified in PDO Évora cheese and were transformed into 4 main fractions



- Up to **35 days of ripening** → increase of the casein degradation rate, remaining relatively constant until the end of maturation (60 days).



- **Urea-PAGE casein degradation** → higher protein degradation of cheeses made with vegetable coagulant than cheeses made with animal coagulant

α_S-caseins = 54.90%	α_S-caseins = 41.86%
β-caseins = 37.27%	β-caseins = 14.95%

- The degradation pattern of caseins → of cheeses made with the diferente *Cynara cardunculus* L. ecotypes was quite similar but strongly differente from cheese made with animal rennet.

Thank you!!!

Questions ?

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